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(54) Title: IDIOTYPIC VACCINE IN LIPID BASED CARRIER FOR B CELL DISORDERS

(57) Abstract

A patient-specific vaccine for non-Hodgkin's lymphoma and other B Cell disorders is made from a lipid-based carrier; and a tumor idiotype derived from B cells from the patient bound to the surface of the lip-based carrier. The idiotype may be absorbed directly to the surface of the lipid-based carrier or bound via an idiotype-binding moiety which is bound to the surface of the lipid-based carrier. When an idiotype binding moiety is used, this moiety is preferably protein G or protein A or a combination thereof. The idiotype need not be prepared from a hybridoma and the use of anti-idiotype murine-derived antibodies is not necessary. Instead, the idiotype can be captured directly from a tumor lysate or other patient-derived material. This makes it possible to prepare a composition in the accordance with the invention and to use it to start the development of an immune response within a period of hours or days, rather than a period of months. The lipid-based carrier is preferably a liposome and may be composed of such lipids as to be an effective adjuvant for the tumor vaccine. The immunotherapeutic composition of the invention can also include additional adjuvants or immunotherapeutic agents incorporated within the liposome. For example, the lipid-based carrier may contain granulocyte-macrophage colony stimulating factor (gmCSF) or cytokines such as interleukin-12 (IL-12) which enhance vaccine-induced immune response.

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IDIOTYPIC VACCINE IN LIPID BASED CARRIER FOR B CELL DISORDERS

DESCRIPTION

FIELD OF THE INVENTION

This application relates to a method and composition for treatment of B cell non-Hodgkin's Lymphoma and other B cell disorders such as chronic lymphocytic leukemia, multiple myeloma and Walderstrom's macroglobinemia.

BACKGROUND OF THE INVENTION

B cell non-Hodgkin's Lymphoma (NHL) is characterized by a clonal proliferation of malignant B cells. One characteristic of most cases of B cell NHL is the expression of a tumor-specific antigen, immunoglobulin, on the cell surface. This surface antigen, termed the tumor idiotype, is composed of variable regions of an immunoglobulin molecule which contain unique determinants, called idiotypes, which themselves can be recognized as antigen.

The expression of a tumor-specific antigen makes B cell non-Hodgkin's lymphoma an excellent target for immunotherapy. The results from previous work have established the feasibility and potential efficacy of passive and active immunotherapy directed against the cell-surface idiotype in the treatment of non-Hodgkin's lymphoma. For example, murine anti-idiotype antibodies to rescued idiotype proteins have been prepared and demonstrate targeting and killing of lymphoma cells with significant clinical responses. Miller, et al., *New Engl. J. Med.* 306: 517-522 (1982). Autologous patient-derived idiotype proteins have also been conjugated with keyhole limpet hemocyanin to produce a vaccine which has demonstrated efficacy and can elicit B and T cell immune responses. Kwak et al., *New Engl. J. Med.* 327: 1209-1215 (1992). More recently, hybridoma-derived idiotype was co-cultured with patient-derived dendritic cells which acted as antigen presenters upon re-infusion into the patient and showed clinical efficacy. Hsu et al., *Nature Medicine* 2: 52-58 (1996). Widespread use of these techniques is limited by current difficulty in the manufacturing, the cost, and the time period (approximately 4-6 months) required to produce these immunotherapeutics.

It is the object of the present invention to provide a composition and method for the treatment of NHL and other B cell disorders which is easily and quickly prepared and which provides effective tumor-specific immunotherapy.

It is a further object of the invention to provide a method for isolation of tumor idiotype and for the use of such idiotype compositions in the treatment of NHL and other B cell disorders.

SUMMARY OF THE INVENTION

These and other objects of the invention are achieved by treating non-Hodgkin's lymphoma and other B Cell disorders in a patient suffering therefrom with a composition comprising:

(a) a lipid-based carrier; and

(b) a tumor idiotype derived from B cells from the patient bound to the surface of the lipid-based carrier. The idiotype may be absorbed directly to the surface of the lipid-based carrier or bound via an idiotype-binding moiety which is bound to the surface of the lipid-based carrier. When an idiotype binding moiety is used, this moiety is preferably protein G or protein A or a combination thereof.

The idiotype can be captured directly from a tumor lysate or other patient-derived material, and does not have to be prepared from a hybridoma and the use of anti-idiotype murine-derived antibodies is not necessary. The invention thus provides a rapid and patient-specific method for isolation of idiotype and preparation of compositions which can be used to promote an immune response against the patient's tumor. The invention makes it possible to prepare such a composition in the accordance with the invention and to use it to initiate the development of an immune response within a period of hours or days, rather than a period of months.

The lipid-based carrier of this invention is selected from the group consisting of micelles, lipoproteins, non-liposomal lipidic complexes and liposomes, e.g., a unilamellar liposome such as a small or large unilamellar liposome or a multilamellar liposome. The lipid-based carrier is preferably a liposome, and may be composed of such lipids as to be an effective adjuvant for the tumor vaccine. The immunotherapeutic composition of the invention can also include additional adjuvants or immunotherapeutic agents incorporated

within the liposome. For example, the lipid-based carrier may contain granulocyte-macrophage colony stimulating factor (gmCSF) or cytokines such as interleukin-12 (IL-12) which enhance vaccine-induced immune response.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a first embodiment of a method for making compositions according to the invention;

Fig. 2 shows a second embodiment of a method for making compositions according to the invention; and

Fig. 3 shows a third embodiment of a method for making compositions according to the invention.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, tumor idiotype from tumor lysates or other patient-derived materials is bound to the surface of a lipid-based carrier molecule either directly or through an idiotype-binding moiety which is bound to the lipid-based carrier to form a vaccine composition. Figs. 1-3 show three different methods for making such a composition schematically.

In Fig. 1, a tumor lysate 10 or other patient-derived material containing tumor idiotype, is purified to isolate the idiotype, I. This can be accomplished rapidly using the methods described in Example 3 and 4 hereof. Basically, this method uses a protein G affinity column or other idiotype-binding solid support to capture idiotype from the tumor lysate and then recovers the separated idiotype by elution. Unlike other methods, this method does not require detergent and can be rapidly accomplished. The purified tumor idiotype, I, is then combined with a lipid-based carrier, LIP, and absorbed on the surface thereof to form an idiotype-lipid-based carrier complex, LIP-I.

Figs 2 and 3 show alternative approaches for making vaccine compositions in accordance with the invention using an intermediate idiotype-binding moiety to link the idiotype to the lipid-based carrier. In one alternative embodiment (Fig. 2), the idiotype-binding moiety is first associated with the lipid-based carrier, LIP, to form the intermediate composition, LIP-IB. As discussed in more detail below, the LIP-IB bond may be covalent or

non-covalent. The LIP-IB is then combined with tumor lysate 10 or to other patient-derived material containing tumor idiotype, with the result that idiotype I is captured by the LIP-IB intermediate to produce the composition LIP-IB-I in accordance with the invention. LIP-IB can also be combined with purified tumor idiotype to form the LIP-IB-I composition of the invention.

In another alternative embodiment (Fig. 3), the idiotype-binding moiety IB is added to tumor lysate 10 or other patient-derived material containing tumor idiotype prior to association with the lipid-based carrier, thus forming the intermediate composition IB-I. This composition is then captured by binding to a lipid-based carrier to form a LIP-IB-I composition according to the invention.

Idiotype-lipid-based carrier compositions made by any of these methods are then used as a tumor-specific therapeutic vaccine for the patient from which the tumor idiotype was derived.

To form an idiotype-lipid-based carrier complex suitable for use in the present invention, the lipid-based carrier needs to absorb or bind to the idiotype with a sufficiently high affinity to provide for rapid binding of idiotype to the lipid-based carrier complex in the first instance, and low release of idiotype from the lipid-based carrier complex during vaccine use. When utilizing a purified tumor idiotype, this can be accomplished in some cases using the inherent protein absorption capabilities of the lipid-based carrier. When a stronger bond between the lipid-based carrier and the idiotype is desired, however, or when the tumor idiotype is being captured directly from tumor lysate or other patient-derived materials without prior purification, an idiotype-binding moiety is used.

Isolation of Idiotype

Isolation of idiotype for use in the embodiment of the invention depicts in Fig. 1 can be accomplished using traditional means, which recover idiotype from hybridomas, serum samples or ascites fluid, as described in Erntell, et al., *Molec. Immunol.* 25: 121-126 (1988), Walker et al., *J. Cell. Biol.* 109(4), Part 2, 307A (1989) and Kwak et al., *N. Engl. J. Med.* 327: 1209 (1992). Preferably, however, isolation of tumor idiotype is accomplished using a new rapid technique which is itself a separate aspect of the present invention. This method is illustrated in detail in Examples 3 and 4. Basically, the method utilizes a solid

support having an idiotype-binding moiety affixed to the surface thereof to capture idiotype from a tumor lysate. The solid support is then washed, to separate unbound materials, and the idiotype is eluted. Example of solid supports which can be used include affinity columns or solid beads, such as for example magnetic beads, having idiotype-binding moieties attached thereto. Suitable idiotype-binding moieties include protein G, protein A and anti-idiotype antibodies which will specifically bind idiotype to separate idiotype from a tumor lysate or other patient derived sample.

Idiotype-Binding Moieties

Because the vast majority of NHL tumor-derived idiotypes are IgM or IgG, lipid-based carriers covalently or non-covalently coupled to molecules which bind to IgM or IgG are suitable for use as idiotype-binding moieties in the present invention. Such molecules include bacterial proteins which bind to immunoglobulins with high affinity. Specific examples of such molecules are Group C Streptococcal protein G ("protein G") or its isolated immunoglobulin binding domain which bind to all isotypes of IgG with high affinity, and Staphylococcal protein A ("protein A") or its isolated immunoglobulin binding domain which bind to the VH3 domain of the IgM molecule. The VH3 domain is utilized in approximately 45 percent of B cells and 40 percent of B cell follicular lymphomas.

Lipid-Based Carrier

As used in the specification and claims of this application, the term "lipid-based carriers" refers to structures primarily composed of one or more lipids, such as the amphipathic phospholipids, which can, but are not required to contain lipid layers and enclosed aqueous volume. Suitable carriers include, without limitation nonliposomal lipid complexes, lipoproteins, micelles and liposomes.

The preferred lipid-based carriers for use in the present invention are liposomes. "Liposomes" are self-assembling structures comprising one or more lipid bilayers, each of which surrounds an aqueous compartment and comprises two opposing monolayers of amphipathic lipid molecules. Amphipathic lipids comprise a polar (hydrophilic) headgroup region covalently linked to one or two non-polar (hydrophobic) acyl chains. Energetically unfavorable contacts between the hydrophobic acyl chains and the aqueous medium are

generally believed to induce lipid molecules to rearrange such that the polar headgroups are oriented towards the aqueous medium while the acyl chains reorient towards the interior of the bilayer. An energetically stable structure is formed in which the acyl chains are effectively shielded from coming into contact with the aqueous medium. Liposomes useful in this invention can have a single lipid bilayer (unilamellar liposomes, "ULVs"), or multiple lipid bilayers (multilamellar liposomes, "MLVs").

Liposomes can be made by a variety of methods (for a review, see, for example, Deamer and Uster, "Liposome Preparation: Methods and Mechanisms", in *Liposomes*, ed. M. Ostro, Marcel Dekker, Inc. NY, pp. 27-51 (1983)). These methods include without limitation: Bangham's methods for making multilamellar liposomes (MLVs), *J. Mol. Biol.*, 13: 238-252 (1965); Lenk's, Fountain's and Cullis' methods for making MLVs with substantially equal interlamellar solute distribution (see, for example, U.S. Patent Nos. 4,522,803, 4,588,578, 5,030,453, 5,169,637 and 4,975,282); and Papahadjopoulos et al.'s reverse-phase evaporation method (U.S. Patent No. 4,235,871) for preparing oligolamellar liposomes. ULVs can be produced from MLVs by such methods as sonication (see Papahadjopoulos et al., *Biochim. Biophys. Acta* 135: 624-638 (1967)) or extrusion (U.S. Patent No. 5,008,050 and U.S. Patent No. 5,059,421). The liposomes of this invention can be produced by the methods of any of these disclosures, the contents of which are incorporated herein by reference.

Various methodologies, such as sonication, homogenization, French Press application and milling can be used to prepare liposomes of a smaller size from larger liposomes. Extrusion (see U.S. Patent No. 5,008,050) can be used to size reduce liposomes, that is to produce liposomes having a predetermined mean size by forcing the liposomes, under pressure, through filter pores of a defined, selected size. Tangential flow filtration (see WO89/008846), can also be used to regularize the size of liposomes, that is, to produce liposomes having a population of liposomes having less size heterogeneity, and a more homogeneous, defined size distribution. The contents of these documents are incorporated herein by reference. Liposome sizes can also be determined by a number of techniques, such as quasi-elastic light scattering, and with equipment, e.g., NICOMP® particle sizers, well within the possession of ordinarily skilled artisans.

The lipid-based carriers used in the present invention can be made using a variety of amphipathic lipids. These include, without limitation: phospholipids, such as phosphatidylcholines (PCS"), phosphatidylethanolamines ("PEs"), phosphatidylserines ("PS's") and phosphatidylglycerols ("PGs"); sterols, such as cholesterol and alpha-tocopherol; and, glycolipids, e.g., galactolipids and glycosphingolipids. Ordinarily skilled artisans are well aware of the varied properties of each of these types of lipids. For examples, PCS have a neutral charge; as such, when incorporated into liposomes, PCS do not change the overall charge of the vesicles, and hence, their attraction/repulsion to cell surfaces. PEs, because of the nature of their headgroup, tend to adopt hexagonal-phase conformations instead of lamellar strictures, and hence, tend to destabilize bilayers into which they are incorporated. Sterols such as cholesterol tend to rigidify lipid bilayers, and hence, decrease the movement of molecules within the bilayers. Accordingly, ordinarily skilled artisans given the teachings of this invention are well able to assess which types of lipids are most suited for use in connection with particular types of lipid-protein conjugates.

In the present invention, the lipid-based carrier may include one or more materials selected to act as adjuvants to increase the immune response generated by the vaccine composition. For example, lipid-based carriers, including liposomes, can be formulated organic acid-derivatized sterols such as cholesterol hemisuccinate. Such lipids, which are further described in US Patents Nos. 4,721,612 and 5,026,557, which are incorporated herein by reference, may be used as a part of, and even as the principal lipid component of the lipid-based carriers of the invention. Another lipid adjuvant which can be used as an immuno-potentiator is Lipid A, the lipid fraction of endotoxin derived from Gram negative bacteria. See, US Patent No. 5,026,557, which is incorporated herein by reference.

In the present invention, the liposomes employed may be prepared to include one or more "bioactive agents" within the encapsulated volume or coupled to or incorporated in the liposome membrane. Bioactive agents which may be associated with this invention's lipid-based carrier include, but are not limited to: antiviral agents such as acyclovir, zidovudine and the interferons; antibacterial agents such as aminoglycosides, cephalosporins and tetracyclines; antifungal agents such as polyene antibiotics, imidazoles and triazoles; antimetabolic agents such as folic acid, and purine and pyrimidine analogs; antineoplastic agents such as the anthracycline antibiotics and plant alkaloids; sterols such as cholesterol;

carbohydrates, e.g., sugars and starches; amino acids, peptides, proteins such as cell receptor proteins, immunoglobulins, enzymes, hormones, neurotransmitters and glycoproteins; dyes; radiolabels such as radioisotopes and radioisotope-labeled compounds; radiopaque compounds; fluorescent compounds; mydriatic compounds; bronchodilators; local anesthetics; nucleic acid sequences such as messenger RNA, cDNA, genomic DNA and plasmids; bioactive lipids such as ether lipids and ceramides; and the like. Preferred bioactive agents which can be included in or coupled to the lipid-based carrier in the vaccine compositions of the invention are cytokines such as IL-12 or gmCSF which can enhance the vaccine-induced immune response.

Coupling of Idiotype-Binding Moiety to Lipid-Based Carriers

As illustrated in Figs. 2 and 3, coupling of idiotype-binding moiety to lipid-based carriers can be performed before or after complexation of the idiotype-binding moiety with patient-derived idiotype. This coupling may be either covalent or non-covalent.

Techniques for affixing proteins to the exterior of liposomes are known in the art. For example, Heath et.al., *Biochim. Biophys. Acta*, 640:66-81 (1981), which is incorporated herein by reference, describe the covalent attachment of immunoglobulins to liposomes containing glycosphingolipid. Leserman et. al. *Liposome Technology, III*, CRC Press, Inc., California, p. 29-40 (1984); *Nature* 288:602-604 (1980) and Martin et. al., *J. Biol. Chem.* 257:286-288 (1982), also incorporated herein by reference, have described procedures for covalently attaching thiolated IgG or protein A to lipid vesicles, and thiolated antibodies and Fab' fragments to liposomes, respectively.

US Patents Nos. 5,047,245 and 5,059,421, which are incorporated herein by reference, discloses another approach to attachment of proteins to liposomes. In this case, the glycoprotein streptavidin is incorporated into the liposome, either covalently or non-covalently, to provide a binding site for the attachment of a biotinylated protein. Methods for making biotinylated idiotype-binding moieties are known, and include those methods disclosed in the Leserman et al. article cited above. Alternatively, streptavidin derivatives of idiotype-binding moieties can be coupled to avidin-containing lipid-based carriers such as those disclosed in Urdal et al., *J. Biol. Chem.* 255: 10509-10516 (1980) and Huang et al., *Biochim. Biophys. Acta* 716: 140-150 (1982). Streptavidin derivatives of idiotype-binding moieties

may be made by various methods known in the art, including expression of fusion proteins from recombinant DNA constructs encoding both the idiotype-binding moiety. A sandwich can also be formed using biotin-containing lipid-based carriers (formed, e.g, using biotinylated phosphatidylethanolamine) and biotinylated idiotype-binding moiety linked by a bifunctional avidin molecule. See, Ahmad et al, *Cancer Res.* 52: 4817-4820 (1992); Ahmad et al., *Cancer Res.* 43: 1484-1488 (1993).

In the present invention, the liposomes or other lipid-based carrier may be coupled with a molecule which binds to the idiotype with high affinity. Preferred examples of such molecules are protein A, protein G, or derivatives thereof such as truncated forms of the protein G molecule, hyperiodinated protein G truncated protein A, or hyperiodinated protein A, although other molecules found to bind to IgG and/or IgM such as an anti-IgM or anti-IgG antibody or other known immunoglobulin binders could also be used. Combinations of liposomes or other lipid-based carrier coupled to protein A and protein G with each other or with other idiotype-binding proteins can also be used.

Covalent coupling of proteins such as protein A to liposomes or other lipid-based carrier can be accomplished using a using a heterobifunctional reagent such as N-hydroxy-succinimidyl 3-(2-pyridyldithio)propionate (SPDP, Pharmacia). SPDP reacts with primary amino groups on proteins to produce a protein-dithiopyridine which in turn is activated by reduction to the thiol, for example with dithiothreitol. Liposomes or other lipid-based carrier incorporating a dithiopyridine-modified lipid, such as dithiopyridine-phosphatidyl ethanolamine can be covalently coupled to the protein-dithiopyridine. The thiol groups of a protein modified with SPDP, succinimidylacetylthioacetate (SATA) or succinimidylacetylthio-proprionate (SATP) and similar bifunctional reagents can also be coupled directly to the maleimido groups of a maleimide-modified lipid incorporated in a liposome. See, U.S. Patent No. 5,399,331 which is incorporated herein by reference.

Non-covalent coupling of the proteins to liposomes or other lipid-based carrier can also be accomplished by coupling anti-idiotype-binding protein antibodies to the liposome surface. This can be accomplished, for example through a biotin/streptavidin interaction or other binding of an affinity binding pair. Thus, for example, an antibody against protein A or protein G could be incorporated in the lipid-based carrier for non-covalent attachment of the idiotype-binding moiety, provided that such non-covalent attachment does not block the site

of interaction between the idiotype-binding moiety and the idiotype. This indirect approach is particularly suitable if the idiotype is complexed to the idiotype-binding protein prior to coupling with the liposome or other lipid-based carrier.

Preparation of vaccine

The composition of the invention is prepared by absorbing substantially purified tumor idiotype to the lipid-based carrier, or by combining an idiotype-binding moiety, either before or after coupling of the idiotype-binding moiety to a liposome or other lipid-based carrier, with a patient-derived sample in which B cell idiotype is accessible for binding. In the case of NHL, this may be a tumor lysate, while in the case of myeloma, idiotype may be found in patient serum. Rescued idiotype may also be used.

Tumor lysates, when needed, can be prepared from one to several grams of tumor tissue which is disrupted to make the normally membrane bound idiotype accessible for binding. One gram of tumor should yield approximately 10^{14} molecules of immunoglobulin (approximately 25 ug). Disruption may be accomplished by sonication, mechanical homogenization, or chemical lysing agents such as detergents. Alternatively, the vaccine can be produced from viably frozen single cell suspensions which are lysed to render the idiotype accessible.

When the idiotype-binding moiety has been previously coupled to a liposome or other lipid-based carrier, lysed tumor cells, serum or other source of patient-derived idiotype protein are combined with the idiotype-binding moiety-liposome and incubated to bind to the B cell idiotype creating idiotype-lipid-based carrier complexes. After incubation, the idiotype-lipid-based carrier complex is then purified from the patient-derived sample. This can, for example, be accomplished by precipitation in high calcium solutions.

When the idiotype-binding moiety has not been previously coupled to a liposome or other lipid-based carrier, lysed tumor cells, serum or other source of patient-derived idiotype protein are combined with the idiotype-binding moiety and incubated to bind to the B cell idiotype creating idiotype-idiotype-binding moiety complexes. One way in which this could be carried out is by passing a patient-derived sample through an idiotype-binding moiety column, such as commercially available protein A columns, to capture idiotype from the sample, and then eluting the idiotype off with a concentrated protein A eluent. If the

idiotype-binding moiety is bound to the column with a reversible linkage, the idiotype-idiotype-binding moiety complex can be exchanged off of the column, resulting in the elution of a purified idiotype-idiotype-binding moiety complex. This complex is then coupled to a liposome which is adapted to permit such coupling, for example through the incorporation of thiolated or maleimido-lipids, or by coupling of an anti-idiotype-binding moiety antibody. If the idiotype-idiotype-binding moiety complex is not prepurified, or if additional purification is needed after incubation, the idiotype-liposomal complex can be purified, for example by precipitation in high calcium solutions.

Prior to combining the tumor idiotype (either in purified form or as part of a lysate) with the lipid-based carrier or idiotype-binding moiety, flow cytometry can be used to determine heavy chain isotypes and thus the appropriate type of idiotype-binding moiety to incorporate in the vaccine. Hyperiodination assays can also be employed to determine the isotype of the idiotype. Alternatively, liposomes or other lipid-based carrier including a mixture of idiotype-binding moieties may be employed in which case this step may be omitted.

Administration of vaccine

The composition of the invention can administered to the patient from whom the tumor idiotype is derived immediately upon preparation or it may be stored for future use. The composition is preferably administered by intramuscular injection in an amount effective to stimulate a therapeutic immune response. It will be appreciated that this amount may vary with the specific formulation of the composition and in some cases with the patient, and that the determination of the appropriate amount to be administered is a routine matter well within the skill in the art.

Administration of the compositions of the present invention offers numerous advantages over the prior processes for immunotherapy for B cell NHL. First, the invention provides a timely tumor-specific vaccine in which the unique epitopes of a patient's own tumor cells are presented as antigen to elicit an anti-idiotypic immune response. Timeliness is important therapeutically, and the present invention permits treatment of NHL without relying upon a method which takes 4 to 6 months to complete production of a therapeutic product.

Furthermore, utilization of the liposome or the lipid-based carrier makes it possible to deliver cytokines locally to the vaccine microenvironment which can help to

potentiate immunologic responses and facilitate the patient's response to the tumor vaccine. In addition, liposomes or other lipid-based carrier themselves have adjuvant properties which can further potentiate the vaccine-induced immune response.

This application refers to various publication and patents. All of these documents are incorporated herein by reference as though fully set forth herein.

EXAMPLE 1

PREPARATION OF CHS LIPOSOME

Multilamellar liposomes (MLVs) were prepared with the Tris salt of cholesterol hemisuccinate (CHS), as set forth in US Patent No. 4,721,612 at col. 11, line 45 - col. 12, line 3 (Example 6.1 - 6.1.1). These liposomes were then extruded, in succession, through filters of pore sizes: 1000 nm, 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, and 100 nm, so as to obtain liposomes of between 100 nm and 200 nm in size

EXAMPLE 2

PREPARATION OF TUMOR LYSATE

Murine B cell lymphoma A20 cells (American Type Culture Collection) were maintained in RPMI-1640 medium supplemented with 10% FBS. Cells were prepared for injection by rinsing cells in T flasks with PBS, at room temperature, transferring to a centrifuge tube and centrifuging for 300 g for 10 minutes. The cell pellets were washed twice in PBS, then the cells were gently resuspended in PBS for injection into mice. The cells were counted and viability of cells (> 90%) was checked by trypan blue exclusion.

Balb/C mice (5 weeks of age) were inoculated with A20 cells s.c., and then sacrificed after approximately 30 days using CO_2. Skin was removed from s.c. tumor and a scalpel was used to slice into the solid mass. Tumor material was recovered by scraping with a scalpel and place into pre-weighed glass scintillation vials. 5mL of cold PBS was added to each vial, and the samples were placed on ice, and then homogenized with a tissue homogenizer (high speed and about 10 strokes). The supernatant was removed and saved. An additional 5mL cold PBS to each vial with the remaining sample and the sample was homogenized again at high speed for 10 strokes. The supernatant was then returned to the vial

and the contents homogenized again at high speed for 5 strokes. The entire sample is then transferred into a 15mL conical tube and sonicated in cold water for 10min. Centrifugation at 3500rpm for 30min at 4°C was then performed to separate the supernatant from a pellet. The pellet was discarded. The supernatant was then centrifuged at 10,000rpm for 10min, separated from the resulting pellet and diluted 1:1 with basic buffer (pH ~8-9)

EXAMPLE 3

PURIFICATION OF TUMOR IDIOTYPE

Idiotype was purified from a tumor lysate prepared as in Example 2 using a Protein G Affinity Column (Pierce, 2ml column) The column was equilibrated with 5mL of Binding Buffer supplied with the column, prior to running the sample through the column. The column was then washed column with 10mL of Binding Buffer, after which the idiotype is eluted with 6 X 1mL portions of Elution Buffer (acidic buffer. ~ pH 3 provided with the column). Other elution buffers which separate idiotpye from the column may also be employed. Fractions are monitored at 280nm and those fractions with elevated readings (these fractions contain the idiotype) are combined.

The combined fractions are passed through a desalting column (Pierce, 5 ml), that has been previously equilibrated with 10mL of distilled water. The column is eluted with 10 X 1mL portions of distilled water to recover the idiotype. Again, the eluted fractions are monitored at 280nm and those fractions with elevated readings are combined. The amount of idiotype recovered can be quantitated based on the absorption or using ELISA techniques.

EXAMPLE 4

PURIFICATION OF TUMOR IDIOTYPE

Idiotype is purified from a tumor lysate prepared as in Example 2 using Goat anti Mouse Immunoglobulin Dyna-beads. The Goat anti Mouse Dyna-beads are washed twice with 0.1N NaOH, and finally washed with neutral buffer such as PBS. Homogenized tumor sample is then added to the beads, and incubated with mixing for 2hrs. at 4°C. The beads are separated from the supernatant by centrifugation or other appropriate means (for example using a magnet if magnetic beads are employed), and washed twice with a neutral buffer such as PBS. Purified idiotype is eluted from the separated beads by adding 5mL 0.5M glacial

acetic acid. The supernatant containing the idiotype is then separated form the beads, and the pH of the sample is adjusted with 0.1N NaOH to about 6.5 to 7. The amount of idiotype recovered can be quantitated based on UV absorption or using ELISA techniques.

EXAMPLE 5

Balb/c female mice (20 g) were injected IM with CHS alone, CHS mixed with 25 mcg of purified A20 murine monoclonal IgG, 25 mcg purified A20 murine monoclonal IgG alone or PBS control. The animals were administered the vaccine material at day = 0, and day = 14. There were 15 mice in each group and the mice were sacrificed at 2 weeks, 4 weeks, 8 weeks and 12 weeks. A tumor-specific humoral response was observed at 8 and 12 weeks.

EXAMPLE 6

Experiments are ongoing to evaluate the ability of the vaccine to prevent tumor engraftment in the murine host. These experiments are utilizing the murine A20 lymphoma cell line which forms subcutaneous tumors in syngeneic Balb/c mice. Idiotype was purified from A20 tumors harvested from Balb/c mice by the procedures described in Example 4 above. Balb/c female mice (20 g) were administered IP CHS alone, CHS mixed with 25 mcg of purified A20-derived tumor idiotype, 25 mcg purified A20-derived tumor idiotype alone or PBS control. The animals were injected IP with the vaccine on day 0 and day 21 and tumor is introduced ip on day 28. Vaccine-treated mice are expected to exhibit increased survival time relative controls or tumor rejection with proper dosage scheduling.

CLAIMS

1. A composition for treatment of cancerous B cell disorders in a patient suffering therefrom, comprising

(a) a lipid-based carrier; and

(b) a tumor idiotype derived from cancerous B cells from the patient bound to the lipid-based carrier.

2. The composition according to claim 1, wherein the tumor idiotype is absorbed directly to the lipid-based carrier.

3. The composition according to claim 1, wherein the tumor idiotype is bound to the lipid-based carrier via an intermediate idiotype-binding moiety.

4. The composition according to claim 3, wherein the idiotype-binding moiety is covalently coupled to the lipid-based carrier.

5. The composition according to claim 3, wherein the idiotype-binding moiety is non-covalently coupled to the lipid-based carrier.

6. The composition according to claim 3, wherein the idiotype-binding moiety is Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

7. The composition according to claim 3, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

8. The composition of any of claims 1 to 7, wherein the lipid-based carrier is a liposome.

9. The composition according to claim 8, wherein the liposome has a cytokine effective to enhance vaccine-induced immune responses included therein.

10. The composition according to any of claims 1 through 7, wherein the patient suffers from non-Hodgkin's lymphoma.

11. A method for forming a vaccine against cancerous B-cell disorder for treatment of a patient suffering therefrom, comprising the steps of:

(a) obtaining a patient-derived sample comprising B cell tumor idiotype;

(b) isolating the tumor idiotype; and

(c) binding the isolated tumor idiotype to the surface of lipid-based carrier to form a lipid-based carrier-idiotype vaccine.

12. The method of claim 11, wherein the tumor idiotype is isolated by separation on a protein G affinity column.

13. The method of claim 11 or 12, wherein the idiotype is absorbed directly to surface of the lipid-based carrier.

14. The method of claim 13, wherein the lipid based carrier is a cholesterol hemisuccinate liposome.

15. A method for forming a vaccine against cancerous B-cell disorders for treatment of a patient suffering therefrom, comprising the steps of:

(a) obtaining a patient-derived sample comprising B cell tumor idiotype which is accessible for binding to an idiotype-binding moiety;

(b) incubating the sample with a composition comprising a lipid-based carrier coupled to an idiotype-binding moiety to form idiotype-lipid-based carrier compositions; and

(c) isolating the idiotype-lipid-based carrier compositions for use as a vaccine.

16. The method of claim 15, wherein the lipid-based carrier is a liposome.

17. The method of claim 15 or 16, wherein the idiotype-binding moiety is Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

18. The method of claim 15 or 16, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

19. A method for forming a vaccine against cancerous B-cell disorders for treatment of a patient suffering therefrom, comprising the steps of:

(a) obtaining a patient-derived sample comprising B cell tumor idiotype which is accessible for binding to an idiotype-binding moiety;

(b) incubating the sample with an idiotype-binding moiety and then coupling the idiotype-binding moiety to a lipid-based carrier to form idiotype-lipid-based carrier compositions; and

(c) isolating the idiotype-lipid-based carrier compositions for use as a vaccine.

20. The method of claim 19, wherein the lipid-based carrier is a liposome.

21. The method of claim 19 or 20, wherein the idiotype-binding moiety is Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

22. The method of claim 19 or 20, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

23. The method according to any of claims 11, 12, 15, 16, 19 or 20 wherein the patient suffers from non-Hodgkin's lymphoma and the cells are lymphoma cells.

24. The method according to claim 23, wherein the idiotype-binding moiety is Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

25. The method according to claim 23, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

26. The method according to any of claims 11, 12, 15, 16, 19 or 20 wherein the sample comprises lysed tumor cells.

27. The method according to claim 26, wherein the idiotype-binding moiety is Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

28. The method according to claim 26, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

29. The method according to any of claims 15, 16, 19 or 20, further comprising the steps of determining the isotype of the idiotype present in the sample prior to incubating the sample with the idiotype-binding moiety, and selecting the idiotype-binding moiety to bind to the isotype present in the sample.

30. The method according to claim 29, wherein the isotype is determined by flow cytometry or a hyperiodination assay.

31. A method for inducing an immune response against a cancerous B cell condition in a patient suffering from therefrom, comprising the steps of:

(a) obtaining a sample from the patient comprising B cell tumor idiotype;

(b) forming a vaccine composition in which tumor idiotype from the sample is bound to a lipid-based carrier; and

(c) administering a therapeutically effective amount of the idiotype-lipid-based carrier composition to the patient.

32. The method according to claim 31, wherein the vaccine composition is formed in accordance with the methods of any of claims 11 through 30.

33. The method according to claim 31, wherein the vaccine composition administered is in accordance with any of claims 1 through 5.

34. The method according claim 33, wherein the lipid-based carrier is a liposome.

35. A method of isolating idiotype from a patient-derived sample containing idiotype, comprising the steps of:

combining the sample with a solid support having an idiotype-binding moiety affixed to the surface thereof to capture idiotype;

washing the solid support to separate unbound materials; and

eluting the idiotype from the washed support.

36. The method of claim 35, wherein the idiotype-binding moiey is protein G Group C Streptococcal protein G or an idiotype-binding subunit or derivative thereof.

37. The method according to claim 35, wherein the idiotype-binding moiety is Staphylococcal protein A or an idiotype-binding subunit or derivative thereof.

38. The method according to claim 35, wherein the idiotype-binding moiety is an anti-idiotype antibody which will specifically bind idiotype.

39. The method according to any of claims 35 to 38, wherein the patient-derived sample is a tumor sample containing tumor idiotype.

40. The method according to claim 39, wherein the patient suffers from non-Hodgkin's lymphoma and the tumor cells are cancerous B cells.

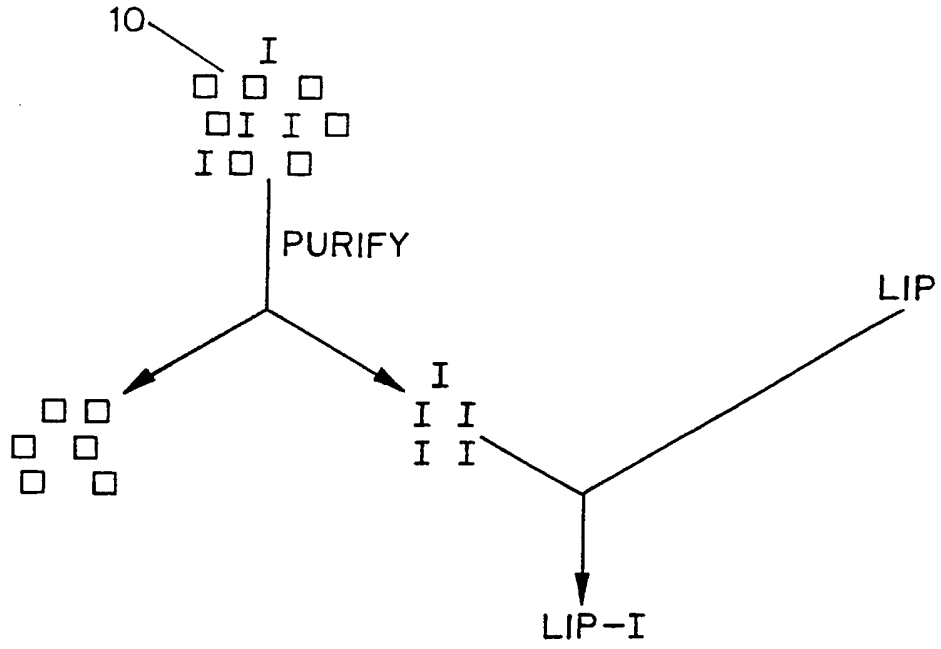

FIG. 1

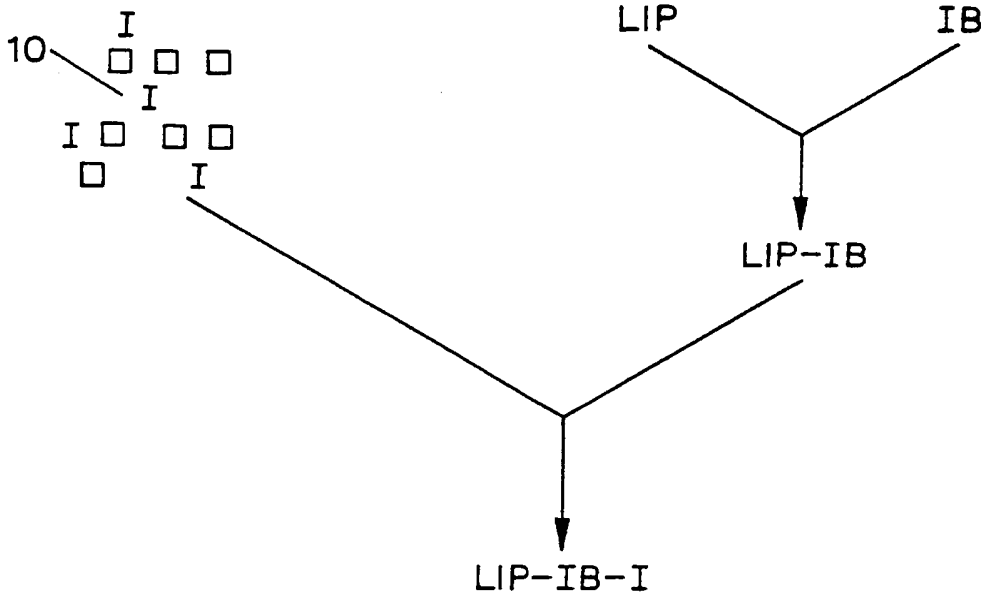

FIG. 2

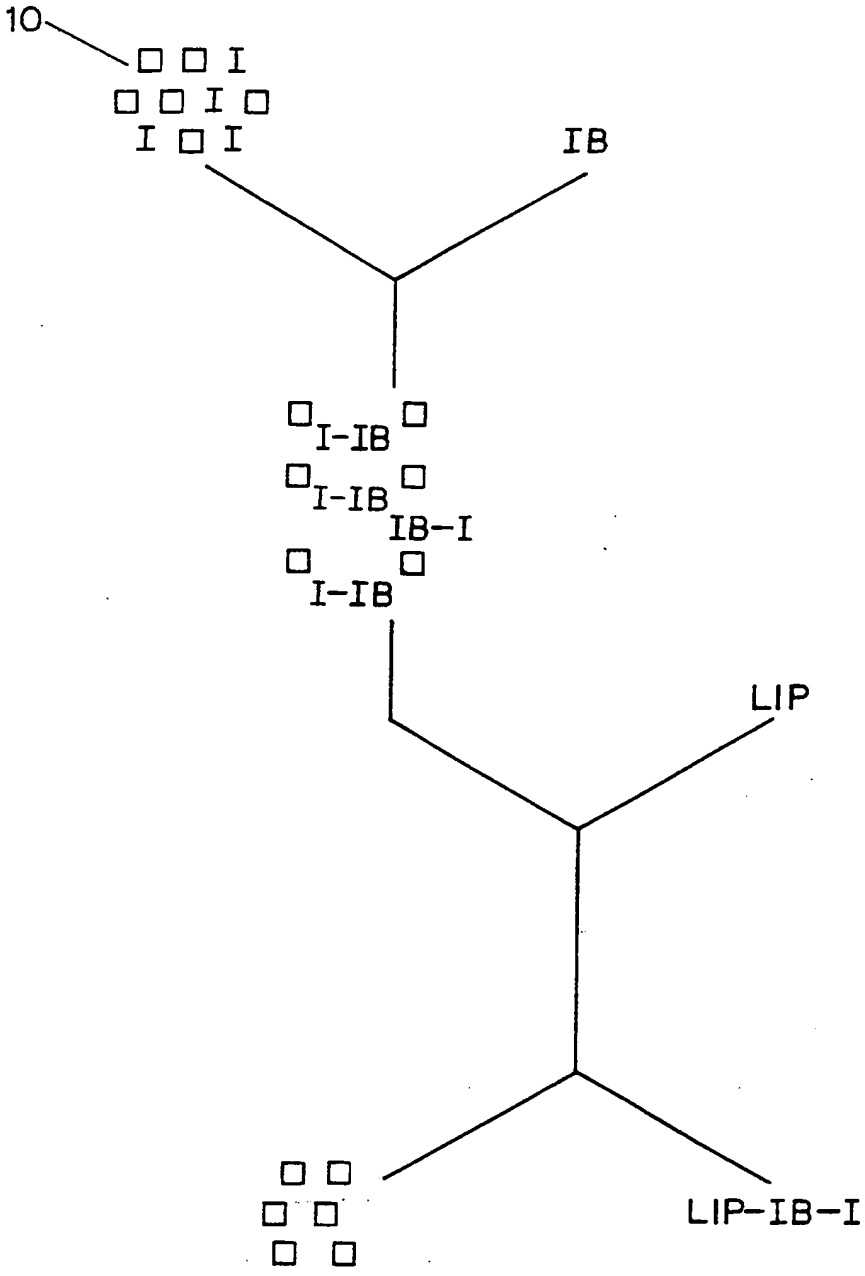

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17513

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 9/127, 39/395
US CL :424/131.1, 450, 172.1, 173.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/131.1, 450, 172.1, 173.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, Biosis, Scisearch, Embase, Cancerlit, WPIDS
search terms: anti-idiotype, B cell lymphoma/disorder, liposomes, protein A and G,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database EMBASE, Abstract No. 83201557, EPSTEIN et al. 'Clinical Consequences of Epstein-Barr Virus Infection and Possible Control by an Anti-Viral Vaccine', abstract, Clin. Exp. Immunol. 1993, Vol. 53, No. 2, pages 257-271, see entire abstract.	1-40
Y	Database MEDLINE on Dialog, US National Library of Medicine, (Bethesda, MD, USA), Abstract No. 95219329, GREGORIADIS, G. 'The Immunological Adjuvant and Vaccine Carrier Properties of Liposomes', abstract, J. Drug Targeting. 1994, Vol. 2, No. 5, pages 351-356, see entire abstract.	1-40
Y	GEORGE et al. Idiotypic Vaccination as a Treatment for a B Cell Lymphoma. J. Immunol. September 1988, Vol. 141, No. 6, pages 2168-2174, especially abstract and discussion.	1-40

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17513

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HARLOW et al. Antibodies, A Laboratory Manual. Cold Spring Harbor Laboratory. 1988, pages 613, 615-618, and 622-623, see entire document.	1-40
Y, P	Database Medline on Dialog, US National Library of Medicine, (Bethesda, MD, USA), Abstract No. 97008117, KWAK et al. 'Vaccination of a Syngeneic, Lymphoma-Derived Immunoglobulin Idiotype Combined with Granulocyte/Macrophage Colony-Stimulating Factor Primes Mice for a Protective T-cell response. Proc. Natl. Acad. Sci. USA. 01 October 1996, Vol. 93, No. 20, pages 10972-10977, see entire abstract.	9